double bonds were correct for palustric acid, one would expect to find bands due to the *cis*-disubstituted double bond between atoms 5 and 6 in its spectrum.

Experimental. The palustric acid employed in the study was kindly sent to the author by E. L. Patton, Head of the Naval Stores Research Section, Naval Stores Section, The United States Department of Agriculture.

The absorption spectrum was recorded with an automatic double-beam spectrophotometer of the type described by Hornig, Hyde and Adcock [6]. The sample was examined using the potassium bromide technique. For further informations, see Ref.[3].

Acknowledgements. I wish to express my sincere gratitude to Dr. Ingrid Fischmeister of the Department of Medical Biochemistry for the spectrophotometric measurements and to Professor Einar Stenhagen for placing the facilities of the Department at my disposal.

1. Loeblich, D., Baldwin, D. E. and Lawrence, R. V. *J. Am. Chem. Soc.* **77** (1955) 2823.
2. Loeblich, D. Baldwin, D. E. and Lawrence, R. V. *J. Am. Chem. Soc.* **77** (1955) 6311.
3. Bruun, H. H. *Paper and Timber, Finland* **38** (1956) 577.
4. Bruun, H. H. *Paper and Timber, Finland* **39** (1957).
5. Bruun, H. H. *Acta Chem. Scand.* **10** (1956) 481.
6. Hornig, D. F., Hyde, G. E. and Adcock, W. A. *J. Opt. Soc. Amer.* **40** (1950) 498.

Received May 31, 1957.

The Formation of Anhydro Vitamin A_1 in the Study of Vitamin A_1 Isomers

B. BARNHOLDT

Statens vitamin-laboratorium, Copenhagen, Denmark

As described by Edisbury *et al.*[1] and later by Meunier *et al.*[2] and Schantz *et al.*[3] the action of an anhydrous (N/30) ethanolic solution of hydrogen chloride on vitamin A_1 results in the conversion of the vitamin into the hydrocarbon anhydro vitamin A_1. Robeson and Baxter [4] have shown that the two isomers 13-*cis* and all-*trans* vitamin A_1 form the same substance when treated with hydrogen chloride in ethanol, but the rate of reaction (in 0.02 N ethanolic hydrogen chloride) is not the same in the two cases.

During recent years a number of new vitamin A_1 isomers [5-7] have been added to those already known, and when faced with the problem of identifying the individual components — isolated for instance by chromatography — of a mixture of vitamin A_1 isomers, it will be natural, side by side with other identification tests, to utilize the process of anhydro vitamin A_1 formation. The aim of the present work has been to develop a convenient procedure for the formation of anhydro vitamin with a view to the utilisation of this reaction for purposes of identification.

It is possible to follow the course of the reaction by spectrophotometry of the reaction mixture, since the formation of anhydro vitamin A_1 causes an increase in the extinction at wavelengths above approx. 340 mμ. Maximum increase is observed at wavelengths of approx. 350, 368.5 and 390 mμ, *i. e.* very near the three maxima of anhydro vitamin A_1 — 351, 371, 392 mμ[3]. Under the action of the hydrogen chloride solution the process continues, and anhydro vitamin A_1 is converted into *iso*anhydro vitamin A_1 (maxima at approx. 330, 350 and 370 mμ [3,8,9]), causing the maximum at 390 mμ to disappear again. The value of E_{390} measured for the reaction mixture thus does not only comprise the extinction due to the anhydro vitamin A_1, but also the extinction of unconverted vitamin A_1 and *iso*-anhydro vitamin A_1. However, the maximum value of E_{390} will almost exclusively be due to anhydro vitamin A_1.

For the purpose of following the process of anhydro vitamin formation the following two magnitudes may be used: (1) the maximum value of the ratio $E_{390} / E^{\circ}_{max.}$ and (2) the corresponding time $t_{max.\ anh.}$ In the ratio $E_{390} / E^{\circ}_{max.}$ is E_{390} the extinction (cell length 1 cm) of the reaction mixture at 390 mμ. $E^{\circ}_{max.}$ is the extinction (cell length 1 cm) of the reaction mixture being due to vitamin A_1 at zero time, *i. e.* at the moment when the vitamin A_1 solution and the hydrogen chloride solution are mixed. $E^{\circ}_{max.}$ is not measured directly, but is calculated from the value of $E_{max.}$ already measured for the vitamin A_1 solution. The value of these two magnitudes, $E_{390} / E^{\circ}_{max.}$ and $t_{max.\ anh.}$ have been determined for all-

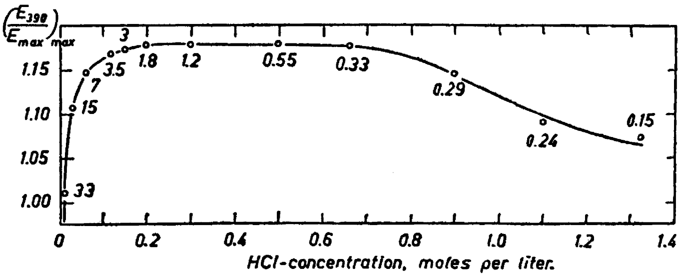

Fig. 1. Maximum values of E_{390}/E°_{325} obtained in the conversion of all-*trans* vitamin A_1 into anhydro vitamin A_1 at different concentrations of hydrogen chloride in ethanol. For each of the experimentally determined points,, the value of $t_{max.\ anh.}$ (in minutes) are stated $t_{max.\ anh.}$ being the time which has elapsed from the moment when the vitamin A solution and the hydrogen chloride solution are mixed and until the reaction mixture exhibits maximum extinction at 390 mμ.

trans vitamin A_1 at different hydrogen chloride concentrations. After mixture of for instance 2.5 ml ethanolic hydrogen chloride solution and 2.5 ml vitamin A_1 solution (containing about 10 μg vitamin A_1 per ml) the reaction mixture was poured into a quartz cuvette, and the course of the reaction was followed by measurements by means of a Beckman DU spectrophotometer. The temperature of the reaction mixture was 25–26°C.

In Fig. 1 the maximum value of the ratio $E_{390} / E^{\circ}_{325}$ and the corresponding reaction time have been represented as a function of the hydrogen chloride concentration in the reaction mixture. It will be seen that $(E_{390} / E^{\circ}_{325})_{max.}$ does not assume values above 1.18. If a further conversion of anhydro vitamin A_1 did not take place, complete conversion of all-*trans* vitamin A_1 into anhydro vitamin A_1 should be expected to result in a value of $\frac{3\,180 \times 268}{1\,850 \times 286} = 1.61$ of $E_{390} / E^{\circ}_{325}$ [E (1 %, 1 cm, 390 mμ) = 3 180[3] of anhydro vitamin A_1, molecular weight 268, E (1 %, 1 cm, 325 mμ) = 1 850[5] of all-*trans* vitamin A_1, molecular weight 268].

When ethanolic hydrogen bromide (0.15 –0.50 N) is used, the same values of $(E_{390} / E^{\circ}_{325})_{max.}$ are obtained as when using 0.15–0.50 N hydrogen chloride. The use of ethanolic sulphuric acid or acetic acid results in lower values of $E_{390} / E^{\circ}_{325}$ and higher $t_{max.\ anh.}$ values than those obtained with hydrogen chloride in the same molar concentration. Even 50 % (v/v) acetic acid in ethanol results in a considerably slower rate of reaction with a lower value of $E_{390} / E^{\circ}_{325}$ than that obtained with, for instance, 0.01 N ethanolic hydrogen chloride. If anhydro vitamin A_1 is formed in a mixture of 1 volume of sulphuric acid (95–97 %) and 9 volumes of absolute ethanol, $t_{max.\ anh.}$ is found to be about 1.0 min, while $E_{390} / E^{\circ}_{325}$ reaches a value of 1.04 only. Lower concentrations of sulphuric acid result in higher values of $t_{max.\ anh.}$ and lower values of $E_{390} / E^{\circ}_{325}$.

The course of the dehydration reaction for all-*trans* vitamin A_1 dissolved in ethanolic p-toluenesulphonic acid has also been examined. Schantz[10] used p-toluenesulphonic acid as catalyst in the preparation of anhydro vitamin A_1. A 10 % (w/v) solution of p-toluenesulphonic acid hydrate (anhydrous p-toluenesulphonic acid was not available commercially) in absolute ethanol gives $t_{max.\ anh.}$ = about 1 min, while $E_{390} / E^{\circ}_{325}$ reaches a value of about 0.5 only. Reduction of the p-toluenesulphonic acid concentration changes the course of the reaction in the same way as decribed with respect to the other acids.

To prepare anhydro vitamin in ethanolic solution at room temperature hydrogen chloride thus seems to be the most suitable of the dehydration agents examined in the present investigation. It may be added that the presence of merely a few per cent of water in the hydrogen chloride mixture will cause a considerable increase in $t_{max.\ anh.}$ and reduce the maximum value of $E_{390} / E^{\circ}_{max.}$.

The influence of variations in the vitamin A_1 concentration on $t_{max.\ anh.}$ and $E_{390} / E^{\circ}_{max.}$ at constant hydrogen chloride concentration has been examined for all-

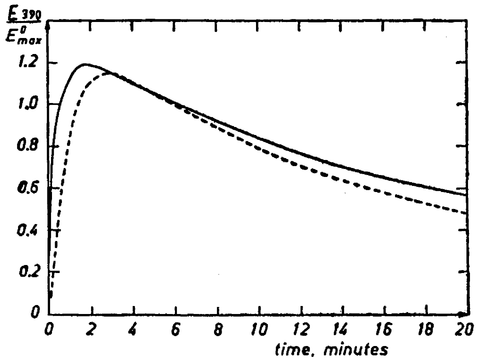

Fig. 2. Conversion of all-*trans* (———) and 13-*cis* (— — — —) vitamin A_1 into anhydro vitamin A_1 in anhydrous 0.20 N ethanolic hydrogen chloride. The graph represents $E_{390}/E^{\circ}_{max.}$ plotted against time.

trans and 13-*cis* vitamin A_1 in 0.20 N ethanolic hydrochloric acid, and both magnitudes were found to be independent of $E^{\circ}_{max.}$ in a concentration range corresponding to $E^{\circ}_{max.} = 0.1-1$.

Fig. 2 illustrates the difference in the dehydration reactions in the case of all-*trans* vitamin A_1 and 13-*cis* vitamin A_1, respectively, in 0.20 N ethanolic hydrochloric acid. It must be considered probable that the magnitudes $t_{max.\ anh.}$ and the maximum values of $E_{390} / E^{\circ}_{max.}$ for the different vitamin A_1 isomers will depend on the number of *cis* configurations and their positions in the molecule and on the molar extinction-coefficient of the vitamin A_1 isomer concerned.

An example of the application of the identification test described in the above will be published elsewhere [11].

The author wishes to thank Dr. phil. Willy Hjarde for his very kind interest in this study and for many valuable discussions.

1. Edisbury, J. R., Gillam, A. E., Heilbron, J. M. and Morton, R. A. *Biochem. J.* **26** (1932) 1164.
2. Meunier, P., Dolou, R. and Vinet, A. *Compt. rend.* **216** (1943) 907.
3. Schantz, E. M., Cawley, J. D. and Embree, N. D. *J. Am. Chem. Soc.* **65** (1943) 901.
4. Robeson, C. D. and Baxter, J. G. *J. Am. Chem. Soc.* **69** (1947) 136.
5. Robeson, C. D., Cawley, J. D., Weisler, L., Stern, M. H., Eddinger, C. C. and Chechak, A. J. *J. Am. Chem. Soc.* **77** (1955) 4111.
6. Dieterle, J. M. and Robeson, C. D. *Science* **120** (1954) 219.
7. Wald, G., Brown, P. K. and Hubbard, R. *Proc. Natl. Acad. Sci. U.S.* **41** (1955) 438.
8. Isler, O., Huber, W., Ronco, A. and Kofler, M. *Helv. chim. Acta* **30** (1947) 1911.
9. Oroshnik, W. *Science* **119** (1954) 660.
10. Schantz, E. M. *J. Biol. Chem.* **182** (1950) 515.
11. Barnholdt, B. and Hjarde, W. *Acta Physiol. Scand.* (1957) *In press.*

Received June 7, 1957.

Depolymerization of 1,2-Dithiolane and 1,2-Diselenolane Polymers

GÖRAN BERGSON and GÖRAN CLAESON*

Chemical Institute, University of Uppsala, Uppsala, Sweden

On hydrolysing trimethylendiselenocyanate, according to Hagelberg [1], Morgan and Burstall [2] a yellow powder is formed which was regarded as the monomer of 1,2-diselenolane (1,2-diselena-*cyclo*pentane) by Morgan and Burstall. Since this molecule is of great interest in connection with the spectrochemical investigations of diselenides, which are in progress at this institute by one of us [3], we tried to dissolve it in the common spectroscopic solvents. It was, however, found to be quite insoluble at ordinary temperatures, but when heated to about 60° the yellow powder darkened and went into solution. This solution was yellow and showed an absorption maximum at about 4 500 Å (Fig. 1). The colour persisted for several days at room temperature and then the yellow powder slowly precipitated. These facts indicate that the solution contains the monomer and that the yellow powder is a polymer, which depolymerizes when heated.

The corresponding sulphur compound (m. p. *ca.* 73°) prepared from trimethylendibromide and sodium disulphide [4] was also investigated. It dissolved without

* Both authors contribute equally.